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Von Economo neurons are present in the dorsolateral (dysgranular) prefrontal cortex of humans

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Abstract

Von Economo neurons (VENs), also known as spindle cells, have been described in layer V of the anterior cingulate (BA 24) and frontoinsular cortex (FI) of humans and other great apes. In the present study we used immunohistochemistry against two specific neuronal markers (NeuN and MAP2) in order to establish the presence of these cell types in Brodmann area 9 (BA 9) of the human prefrontal cortex. We evaluated tissue samples of eight human postmortem brains (age range 26–50) from BAs 9, 24, 4, 46, 45, 10 and 17. We identified a group of cells with similar morphology to that previously described for VENs in all specimens of BA 9 examined, albeit less frequently than in BA 24. This is the first description of this cell type in a human brain area with well developed granular layers (BA 9).

Keywords: Von Economo neurons; Spindle cells; Anterior cingulate gyrus; Frontoinsular cortex; Dorsolateral prefrontal cortex

Von Economo neurons (VENS) are bipolar in appearance. Their elongated contour is emphasized by ascending and descending processes generated from opposite extremes of the cell body extending perpendicular to the pial surface. These gradually tapering processes are indistinguishable from the soma [1,2,7,8,21]. Von Economo neurons have large cell bodies; on average, four to five times greater than adjacent layer V pyramidal neurons [7]. Their large cell bodies and equally prominent axons suggest that VENs provide for the rapid transmission of information to other parts of the brain. Both in humans and chimpanzees VENS appear late in gestation (35 and 32 weeks, respectively). In humans the number increases until 4 years, while in chimpanzee VENs decrease in early postnatal life [4].

Initially Von Economo and Koskinas [19] described these cells in layer V of the anterior cingulate (ACC) and frontoinsular cortices (FI). Based on their location it has been proposed that VENs play a role in both emotional and sophisticated cognitive behaviors [2]. It was believed that VENs were present only in great anthropoids and that a direct correlation between encephalization and size/cell density existed. In the frontoinsular cortex they are found in great African apes but not in the orangutan; thus dating their emergence in this region to 9 Mya [20]. Recently they have been described in a more generalized distribution in the brains of large cetaceans where they may have developed independently from primates as an apparent example of convergent evolution [5].

To our knowledge there have been no reports in humans of VENs in regions other than BA 24 and frontoinsular cortex. In the current study, we investigated for the presence of VENs in BAs 4, 9, 10L, 17, 24, 45, and 46 of the human cerebral cortex. Postmortem tissue samples were obtained from eight human subjects from the Instituto Nacional de Medicinal Legal y Ciencias Forenses of Cali, Colombia. The subject's age ranged from 26 to 50 years and the postmortem interval (PMI) was less than 24 h (Table 1). Specimens were selected according to pathological criteria, not including those tissues with evidence of gross or microscopic pathology. The samples were fixed with paraformaldehyde-lysine-periodate (PLP) (pH 7.4) at 4 °C for 7–10 days. The blocks were cut at 50 μm using a vibratome (Lancer Vibratome series 1000®). The first

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Table 1
Description of the cases for the study

Case	Gender	Age (years)	Weight (g)	PMI (h)
1	M	47	1475	24
2	M	26	1375	13
3	M	50	1350	12
4	M	37	1275	8
5	M	47	1350	15
6	M	31	1500	11
7	M	35	1600	12
8	M	48	1500	8

PMI, postmortem interval.

section was stained with toluidine blue to evaluate the proper coronal plane of section and to corroborate cytoarchitectural criteria. In addition, we obtained tissue samples from the anterior cingulate cortex (BA 24) at the level of the genu of the corpus callosum and from BAs 4, 46, 45, 10L and 17. Sections were incubated with neuronal specific antibody (NeuN, Chemicon International—MAB377®) [6] or a somato dendritic marker (anti-MAP2 [5f9] gift of Dr. Kenneth Kosik, LA). The tissue was immersed in 30% methanol with hydrogen peroxide 0.3%. Non-specific binding was blocked with 1.5% normal horse serum (Sigma) and incubated overnight with either NeuN monoclonal antibody (Chemicon International—MAB377®) diluted to 1:100 in PBS Triton X-100 0.5% or with anti-MAP2 diluted to 1:100 in PBS Triton X-100 0.5%. Sections were serially incubated with a biotinylated secondary horse anti-mouse antibody followed with avidin-biotinylated horseradish peroxidase complex. All steps were followed by washes in PBS. Finally, sections were developed in a substrate solution of nickel-enhanced 3-3-3′ diaminobenzidine. The tissue was rinsed in distilled water, mounted in cromoaluminated glass slides, dehydrated and coverslipped. Control sections were subjected to the same procedure but primary antibodies were omitted. Additionally, we performed Nissl staining on 5 μm thick paraffin embedded tissue from all selected areas.

Fig. 1A shows the laminar distribution of NeuN-stained cells in human BA 9. Tissue samples of BA 9 were selected from the medial surface of the superior frontal gyrus, 5 mm ventral to the dorsolateral convexity [11,12]. According to Rajkowska and Goldman Rakic [13] BA 9 is characterized by a wide layer III and a thin layer IV. Additionally, cell density is lower in layers IIIb, Vb and VIb as compared to the other layers with the exception of layer I. In the present study we used these criteria to identify BA 9.

After analyzing BAs 9, 24, 4, 46, 45, 10 and 17, only BAs 24 and 9 had VEN's. A VEN from layer Vb of BA 9 is shown in Fig. 1B. This neuron shows an elongated soma with ascending and descending processes stemming from its polar ends. The axon is not visible because NeuN immunoreactivity is restricted to the soma and dendritic processes. This description fulfils the characteristics described by Nimchinsky et al. [7] for VENs using the Nissl method. The finding of VENs in BA 9 extends Watson and Allman's [20] observation that these cells may reflect a functional specialization of agranular/dysgranular prefrontal cortices.

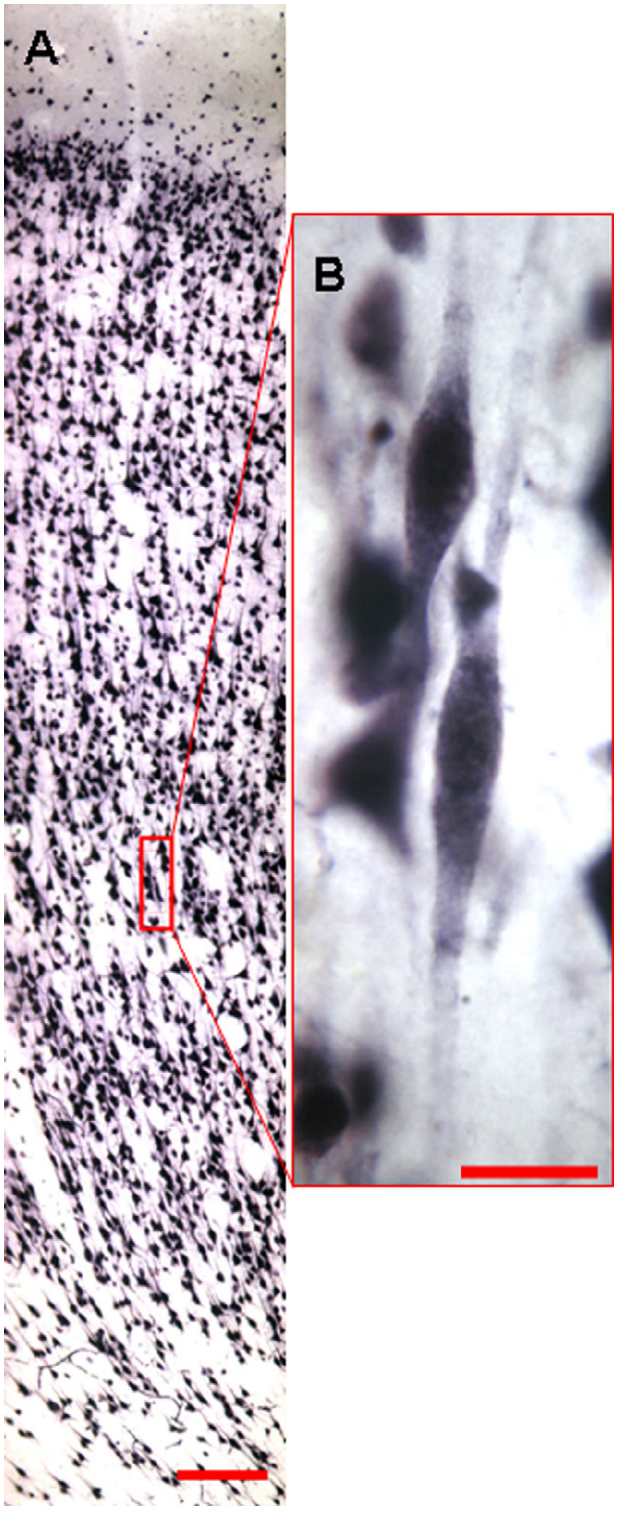

Fig. 1. (A) Laminar distribution of human dorsomedial prefrontal cortex (BA 9) observed by NeuN immunoreactivity and (B) Von Economo neurons observed by NeuN. Scale bar 200 μm (A) and 50 μm (B).

Fig. 2 compares the NeuN, Map 2 (5f9) and Nissl reactivity of VENs in BA 9 and 24. While VENs in BA 24 are numerous [7], their presence in BA 9 is sparse and their density varies from sector to sector. In order to determine the contribution of VENs on BA 9 as compared to BA 24, we counted on layers Va

and Vb the total neuronal population (TN) of VENs present in 12 samples of 4 subjects for each area. Neuronal quantification was made using a box of 500 μm in a tangential plane by the depth of the layer (620.7–787.4 in BA 24 and 469.5–499.3 in BA 9), with the Sigma Scan Pro 5.0 program. Statistical analysis was made using student's *t*-test. The VENs/TN ratio provides a relative indicator of the VENs population. In BA 24, 12 of 12 sections showed VENs while only 10 of 12 sections in BA 9 showed VENs. In our material VENs in BA 24 represent 3% of the total cell number in layer V, while in BA 9, VENs account for 0.5% (taking NeuN positive cells as the universe in both cases). VENs on BA 24 are usually organized in clusters of 2–4 cells; in BA 9 most of them are isolated and occasionally found in clusters of 2 cells. It is important to mention that in BA 9 VENs are discontinuously distributed; being frequently absent in sections adjacent to well populated regions. In plane of sections where dendritic bundles of pyramidal neurons were visible it was possible to appreciate apical processes of VENs joining to dendritic bundles of neighbor pyramidal cells (Fig. 3). In order to determine the area of VENs we measured 50 cells, 25 in BA 24 and 25 in BA 9. It was found that VENs from BA 24 are smaller ($536.3 \pm 161.5\ \mu m^2$) than BA 9 ($627.5 \pm 96.92\ \mu m^2$) ($p < 0.05$). Although we did not found VENs on frontal BA 10L, 46, 45, this observations should be considered preliminary because these areas are extensive and we evaluated only a small sector of them in a limited number of subjects.

This is the first report of VENs in human brain regions other than ACC and frontal insular cortex. We found this particular cellular morphology present in BA 9, although the density and the clustering of theses neurons were reduced as compared to BA 24. However, the morphologic characteristics of these neurons were similar in these two cortical areas. Despite some differences in the functional domains of these cortical areas they are both coactivated in normal and bipolar disorders subjects when performing an interference task [17]. VENs may share a similar specialization by linking established attentional/executive networks with relevant areas of the brain, e.g., sensory, association, limbic. This similarity at the level of connectivity may well be exemplified in the rhesus monkey where BA 24 projects to BA 9 [18] and both project strongly to dorsal hypothalamus and lateral periaqueductal gray [9,10]. However, at present it is difficult to establish the contribution of VENs and other cells of the same region in these networks.

Based on the appearance of spines in Golgi impregnation VENs are excitatory and, along with spiny stellate cells, are considered modified pyramidal neurons [7]. Their narrow den-

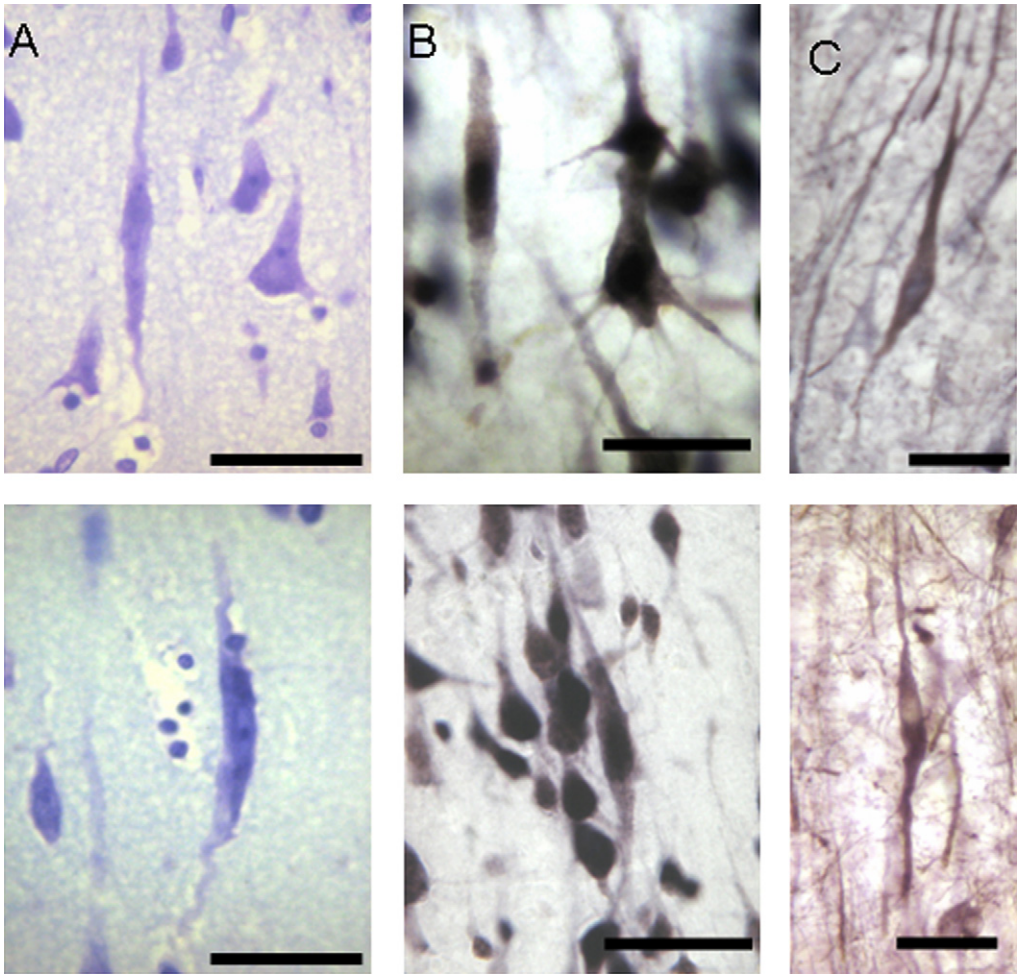

Fig. 2. Comparison of VENs of área 9 (up) and área 24 (down) by the markers Nissl (A), NeuN (B) and Map 2 (C). Scale bar 50 μm.

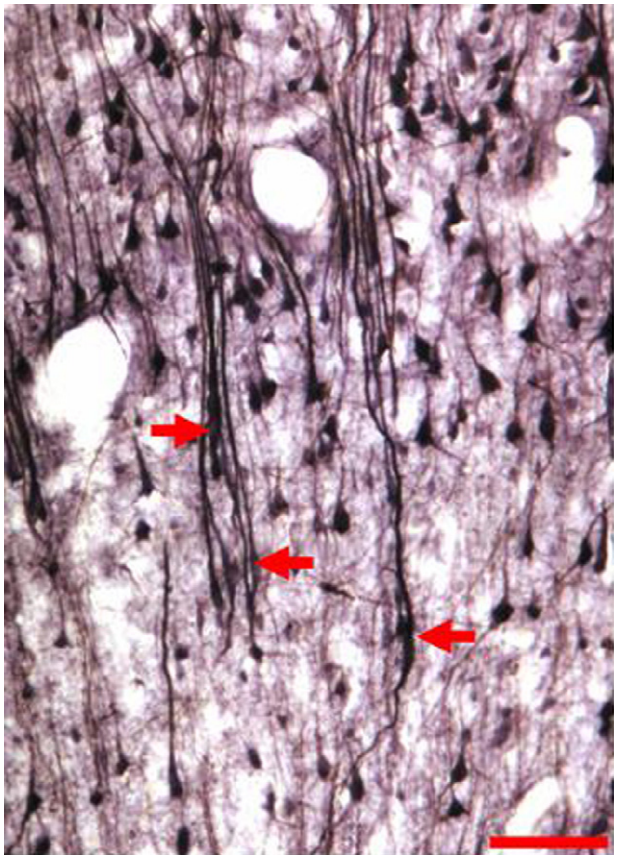

Fig. 3. Von Economo neurons of area 24 integrating in dendritic bundles, arrows indicate Von Economo neurons. Scale bar 100 μm.

dritic tree (35–60 μm) and the integration of apical projections into dendritic bundles originating at layer V has suggested that VENs function within individual minicolumns [20]. This is of some importance as certain conditions characterized as minicolumnopathies have shown regional abnormalities consistent with the topographical distribution of VENs [3]. Moreover the organization of BA 9 is altered in pathologies like schizophrenia [6,16] major depressive disorder [14] and frontotemporal dementia [15] making it of interest to study the expression of VENs in theses conditions.

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